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A Family Inheriting Different Subtypes of Acute Myelogenous Leukemia

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Rare inherited cancer syndromes have proven invaluable for the identification of genes involved in the more frequent corresponding noninherited cases. We report on a family with an adult onset, incompletely penetrant, autosomal dominant syndrome of myelodysplasia and acute myelogenous leukemia, affecting at least eight, and probably ten, individuals from three generations. The patients have developed leukemias differing in morphologic subtype, tumor cytogenetics, and abruptness of presentation. Some have presented with acute onset and others with protracted myelodysplasia. This family does not have an unusual incidence of other malignancies; however, one person at 50% risk of inheriting this gene developed atypical mycobacterium infection in the absence of leukemia, but also without appreciable risk factors for acquired deficiencies in cellular immunity. Features common to affected family members, including the individual with mycobacterium infection, are the early presence in the bone marrow of red cell and platelet maturation defects. A search for mutations in diseased marrows fails to detect abnormalities of *p53* or N-*ras*. Two of the affected family members, third degree relatives, have co-inherited a constitutional chromosomal banding variation of 9p21-22, potentially suggesting linkage to this locus. The variable penetrance and expressivity of this syndrome support a multistep model of leukemia evolution, in which the gene defined by this family's syndrome is the signal step.

Key words: acute myelogenous leukemia, tumor suppressor gene, chromosome 9p, *Mycobacterium avium intracellulare*

INTRODUCTION

Rare families with cancer-predisposing syndromes potentially illuminate the genetic mechanisms of the common isolated occurrences of these tumors. The aberrant genes inherited in the germ line of these uncommon individuals have subsequently been found to be mutated somatically in some sporadic cases [reviewed in 1]. The discovery of most of the known tumor suppressor genes and clinically relevant DNA repair genes originated through family studies.

Familial leukemia is uncommon. A few pedigrees transmitting lymphocytic leukemia, acute and chronic, have been reported [reviewed in 2]. Descriptions of inherited myelogenous leukemia are rarer still. Two families are known with three or more members of a sibship developing acute myelogenous leukemia (AML) [3,4]. Another two families have been described with several cousins affected with AML [5,6]. There are just three prior reports of multigenerational transmission of myelodysplasia and/or AML to three or more members of a family. One of the families [7] manifest solid tumors, in addition to AML. In another family there was myelodysplasia without evolution to leukemia [8]. The third family [9] transmitted AML or a related hematologic malignancy exclusively. Another family has been reported in which there is likely X-linked recessive inheritance of both acute lymphocytic and myelogenous leukemia [10].

We present a family with a syndrome of incompletely penetrant, apparently autosomal dominant, adult-onset

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myelodysplasia and AML. In addition to the large size of this family, it appears unique from the previously reported families in that the leukemias differ in morphologic subtype and other clinical features but have common early histopathological marrow changes involving the red cells and platelets. An additional family member developed an opportunistic infection characteristic of deficiency of cellular immunity. There is not an unusual incidence of other malignancy in this family. A screen for tumor mutations in the N-*ras* protooncogene and the *p53* tumor suppressor gene is negative. Two of the family members have a constitutional cytogenetic variation of chromosome 9p21-22.

MATERIALS AND METHODS

Patients

Informed consent for treatment and relevant additional studies was obtained for cases IV-1, IV-4, and IV-6 at our institutions. Family interviews and civil records were used to delineate the pedigree. Medical records were acquired with consent from surviving kin. Approval from the Fred Hutchinson Cancer Research Center institutional review board was obtained for mutation analysis. In addition to obtaining material from individuals III-1, IV-1, IV-4, and IV-6, archival tissue was procured from the outside institutions for individuals II-2, II-9, III-4, and III-7. We personally reviewed bone marrow aspirations and biopsies on patients II-2, II-9, III-1, III-4, III-7, IV-1, IV-4, and IV-6.

Sample Preparation

Paraffin embedded blocks were cut into 10 mm slices and incubated at 55°C for 2 hr in 200 μl of 10 mM tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20, and 0.1 mg proteinase K. The sample was then boiled for 15 min. Typically, about 100 to 500 ng DNA was recovered per sample. From a total of 24 PCR reactions performed on archival material, amplified product sufficient for SSCP analysis was obtained from 6 reactions.

Polymerase Chain Reaction (PCR) Analysis

Ten microliters of the above isolated DNA was PCR amplified for subsequent single strand conformation polymorphism (SSCP) mutation analysis [11]. Amplification of N-*ras* exon 1 yielded a 115 bp PCR product encompassing codons 12 and 13, while amplification of exon 2 yielded a 111 bp product encompassing codons 59–63 [12]. *p53* exons 5 and 6 were amplified simultaneously, yielding a 408 bp product. For SSCP analysis this product was digested with Hae II, yielding fragments of 230 and 178 bp [13]. Exons 7 and 8 of *p53* were amplified separately, yielding PCR products of 138 and 160 bp, respectivley (14). All PCR amplifications were carried out in a total volume of 50 μl.

One-tenth of the PCR product was diluted 1:10 in 95% formamide, 0.5 M EDTA, and 1% SDS, heated at 95°C for 10 min, then loaded onto a 0.5 × MDE Hydrolink gel (AT Biochem, Malvern, PA). Two SSCP conditions were run: one gel contained 10% glycerol and was run at room temperature, and the other was run at 4°C. DNA obtained from an unaffected bone marrow was used as a negative control in all reactions. Positive control DNA for N-*ras* exon 1 was from THP-1 cells, for N-*ras* exon 2 from HL-60 cells, for *p53* exons 5 and 6 from Jurkat cells, for *p53* exon 7 from Ramos cells, and for *p53* exon 8 from SW480 cells.

RESULTS

Pedigree

The family (Fig. 1) is of northern European ancestry. The affected individuals have resided throughout the United States.

Case IV-1

The proband was a previously well 21-year-old, pregnant at 28 weeks gestation, when she presented in 1992 to the University of Washington Medical Center with constitutional symptoms and a white count of 2.7×10^9/L, hematocrit of 21%, and platelet count of 62×10^9/L. The peripheral smear (Fig. 2) showed anisopoikilocytosis with considerable polychromasia. Nine nucleated red cells were present per 100 white cells counted. Dysmorphic platelets were noted. The white cell differential count included 24% neutrophils, 32% lymphocytes, 3% monocytes, 1% myelocytes, and 36% blasts. The blasts were medium- to large-sized cells with a high nuclear: cytoplasmic ratio, immature chromatin, and prominent nucleoli. A few blasts were smaller in size with cytoplasmic projections, suggestive of micromegakaryocytes. The granulocytes had hypolobated nuclei with abnormal granulation. A marrow aspirate could not be obtained. The bone marrow core biopsy showed marked fibrosis with small dysplastic megakaryocytes as well as immature myeloid cells. A reticulin stain (Fig. 3) demonstrated a marked increase in reticulin. Cytochemical stains were positive for myeloperoxidase in a rare blast. Cell surface marker studies performed by flow cytometry showed that the blasts expressed the myeloid markers CD13 and CD33, as well as the megakaryocytic marker CD41 and the stem cell marker CD34. Cytogenetics performed on peripheral blood revealed nineteen out of twenty metaphases with monosomy 7, and thirteen of these cells had a supernumerary derivative chromosome consisting of translocation of 1q to 7p. One of the latter cells was also trisomic for chromosome 8. A diagnosis of acute megakaryoblastic leukemia was made (FAB type M7). The patient completed induction chemotherapy and was delivered of a healthy daughter (individual V-1) at 33 weeks gestation. Four months later, with refractory persistance of leukemia failing consolidation therapy and an

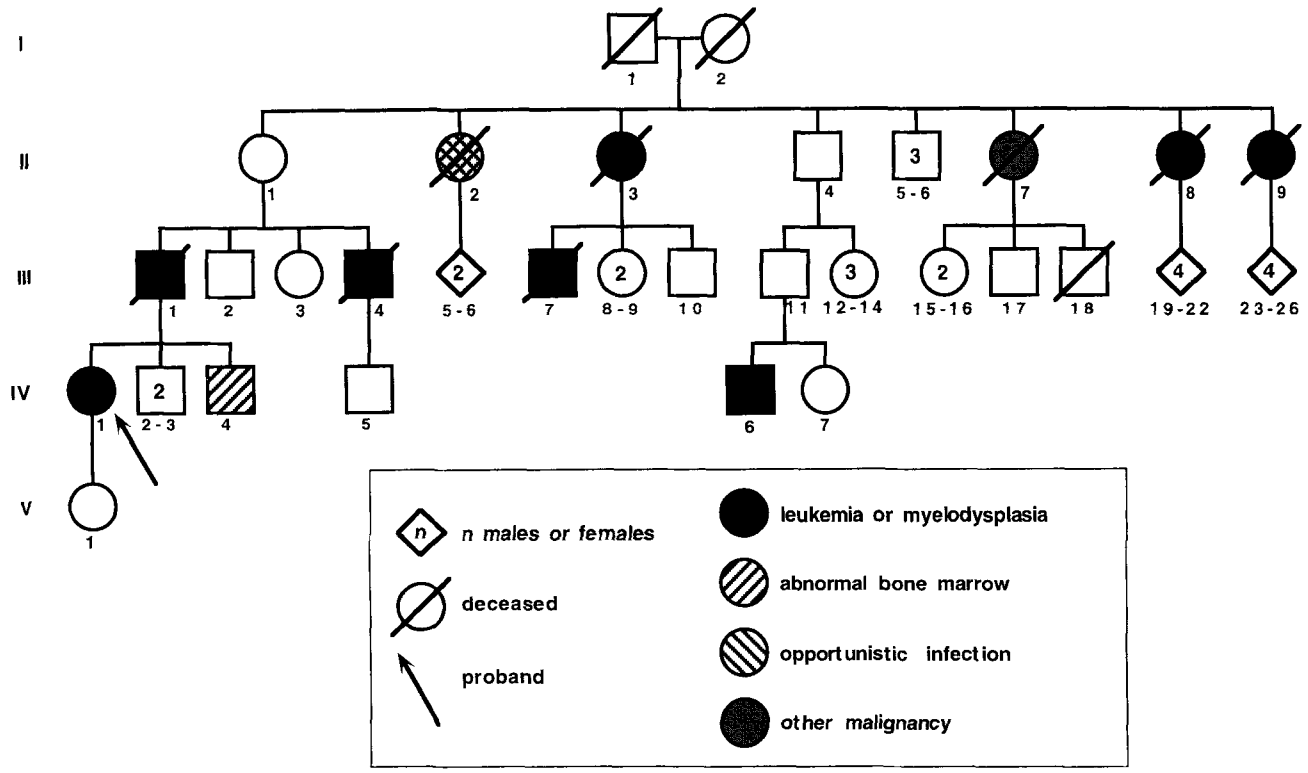

Fig. 1. Pedigree. Only affected individuals and their parents, children, or siblings are shown.

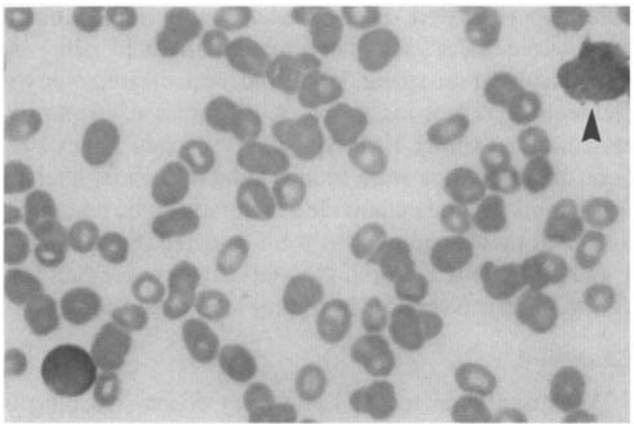

Fig. 2. Case IV-1, peripheral blood smear, ×500, Wright-Giemsa stain. In addition to marked red cell anisopoikilocytosis, two blasts are present, one of which (arrowhead) has small cytoplasmic projections suggestive of micromegakaryocyte morphology.

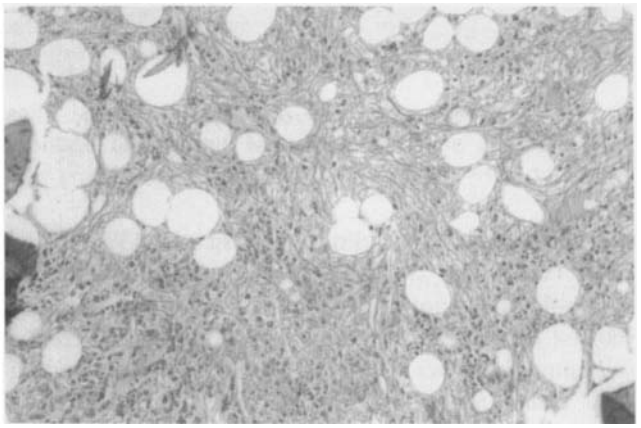

Fig. 3. Case IV-1, bone marrow core biopsy, ×100, reticulin stain. Normal marrow architecture is absent, and there is a marked increase in reticulin, consistent with extensive fibrosis.

unsuccessful search for unrelated donors, she received an HLA-matched transplant from her brother. PCR with SSCP analysis of her leukemic marrow found no detectable mutation in N-*ras* exons 1 and 2 and *p53* exons 5 through 8.

Case IV-4

The proband's brother, a 16-year-old in good health, was evaluated in 1993 at the Fred Hutchinson Cancer Research Center as a possible allogeneic bone marrow donor for his HLA-matched sibling. The patient was taking no medications at the time of evaluation. The white

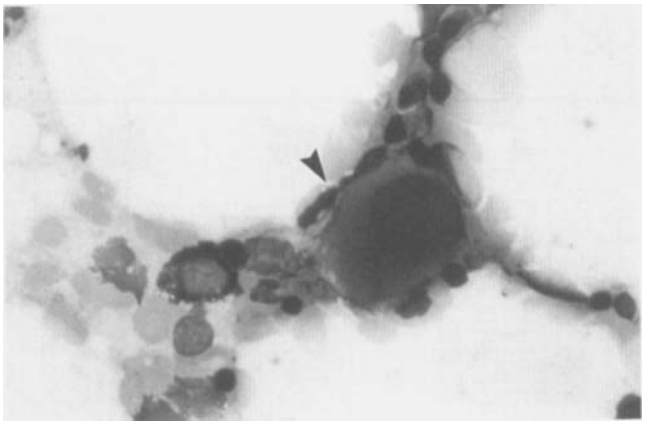

Fig. 4. Case IV-4, bone marrow aspirate smear, ×500, Wright-Giemsa stain. An abnormal megakaryocyte is shown (arrowhead).

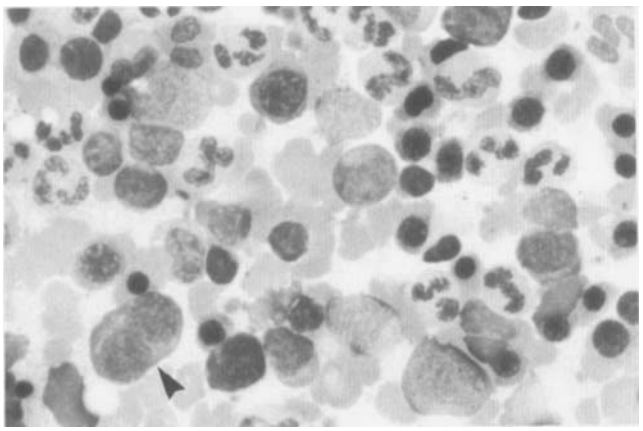

Fig. 5. Case IV-4, bone marrow aspirate smear, ×500, Wright-Giemsa stain. An abnormal binucleated erythroid precursor cell is indicated (arrowhead).

count was 6.0 × 10^9/L, the hematocrit was 43%, and the platelet count was 215 × 10^9/L. The peripheral blood was morphologically normal. The bone marrow aspirate smear showed trilineage maturation with a normal differential cell count; however, rare cells with cytoplasmic and nuclear characteristics of small hypolobated megakaryocytes could be identified (Fig. 4), and rare binucleated erythroid cells (Fig. 5) could be found with extensive searching. The bone marrow core biopsy showed normal cellularity. Cells with a morphology consistent with hypolobated megakaryocytes could be identified in the biopsy as well. Marrow cytogenetic analysis was normal. No marrow mutation of N-*ras* exons 1 and 2 and *p53* exons 5 through 8 was detected.

Case III-1

The proband's father was a 41-year-old treated at the University of Washington Hospital in 1979. He had a four-year history of pancytopenia requiring 3 to 4 U of packed red cells per month. The white count had ranged from 1.8–2.3 × 10^9/L, the platelet count decreased from 208 × 10^9/L to 28 × 10^9/L during this time. Up to 10–15% blasts had been noted in the peripheral blood. Four months prior to admission the white count increased to 31 × 10^9/L, and the platelet count rose to 162 × 10^9/L, with no improvement in the red cell count. The patient's course had been complicated by frequent infections and fever. Other than transfusions, the patient had received no medication prior to admission. On admission the white count was 20 × 10^9/L, the hematocrit was 26.8%, and the platelet count was 14 × 10^9/L. The peripheral blood showed mild anisopoikilocytosis, markedly decreased platelets, and a mild leukocytosis consisting of 32% blasts, 2% myelocytes, 45% neutrophils, 19% monocytes, and 2% lymphocytes. The blasts had a high nuclear:cytoplasmic ratio and fine chromatin. Some of the blasts had increased cytoplasm and folded nuclei, giving a monocytoid appearance. The neutrophils showed dysplastic features including both hypersegmented and hyposegmented nuclei; the monocytes also had a abnormal appearance with nuclear immaturity. The bone marrow aspirate smear contained large numbers of monocytoid blasts, as well as some residual normal neutrophils and monocytes (Fig. 6). Megakaryocytes and erythroid cells were virtually absent, although rare scattered micromegakaryocytes could be identified. There was increased storage iron. Cytochemical stains were positive for myeloperoxidase and negative for nonspecific esterases. The bone marrow core biopsy showed 100% cellularity with large numbers of myeloid blasts and more mature myeloid cells. As with the aspirate smear, erythroid and megakaryocytic elements were not identified. A diagnosis of acute myelogenous leukemia, FAB type M2, was made. No cytogenetic abnormalities were found. Induction chemotherapy was begun, but the patient died of hemorrhagic complications. No marrow mutations were detected in N-*ras* exon 1 and *p53* exons 7 and 8. No amplification product was detectable for N-*ras* exon 2 and *p53* exons 5 and 6.

Case III-4

At presentation to the Latter Day Saints Hospital in Salt Lake City in 1971, the paternal uncle of the proband was a 28-year-old with a 5-year history of transfusion-dependent pancytopenia. One month prior to admission the patient was diagnosed with acute myelogenous leukemia, complicated by parotitis and thrombocytopenia. The original diagnostic material was not available for review. At admission the patient had a white count of 1.2 × 10^9/L, a hemoglobin of 67 g/L, and a platelet count of 10 × 10^9/L. A bone marrow aspirate smear obtained after the initiation of chemotherapy showed a paucity of cellular elements. A differential cell count included 26% blasts, 4% promyelocytes, 1% metamyelocytes, 1% bands, 12%

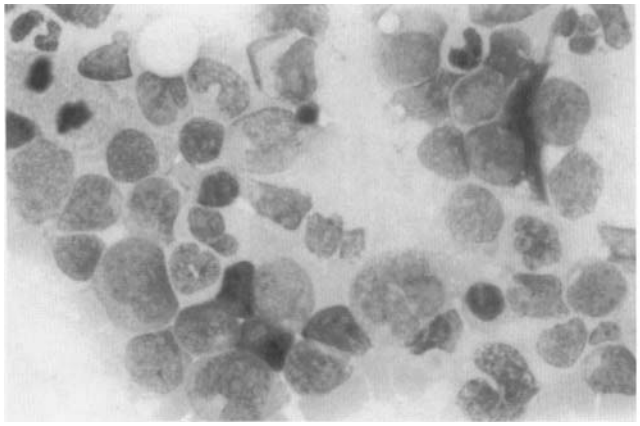

Fig. 6. Case III-1, bone marrow aspirate smear, ×500, Wright-Giemsa stain. Increased numbers of myeloblasts are evident.

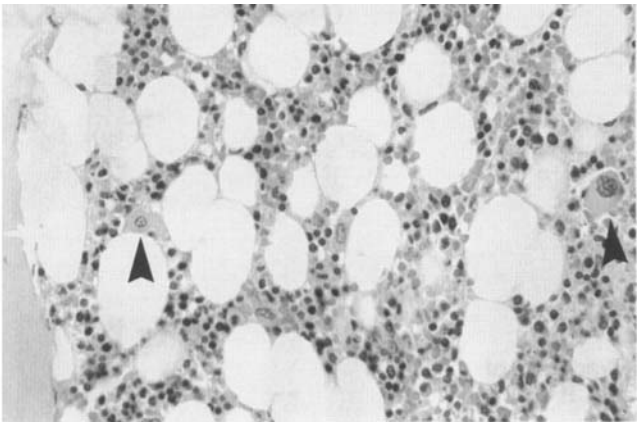

Fig. 7. Case IV-6, bone marrow core biopsy, ×100, Wright-Giemsa stain. The marrow is hypocellular with mild megaloblastic changes in the erythroid series. Two small hypolobated megakaryocytes are present (arrowheads).

eosinophils, and 56% small lymphocytes. No normal erythroid precursor cells or megakaryocytes were seen. There were scattered hemosiderin-laden macrophages, consistent with a long history of red cell transfusions. No core biopsy or peripheral blood smear were available for evaluation. The bone marrow aspirate smear was consistent with a diagnosis of acute myelogenous leukemia in relapse; no FAB classification was possible. The post-chemotherapy course was complicated by persistent pancytopenia with bleeding episodes and sepsis, leading eventually to the patient's death. PCR amplification of archival marrow was unsuccessful.

Case II-9

The maternal aunt of the prior two cases was 32 years old at the time of presentation to the University of Minnesota Hospital in 1967. There was a lifelong history of easy bruising. In the time leading up to admission the patient had 1 year of increasing fatigue, 2 months of menorrhagia, and 4 days of right lower extremity cellulitis/thrombophlebitis. On admission the hemoglobin was 66 g/L, the platelet count was 76×10^9/L, and the white count was 1.3×10^9/L, consisting of 6.5% blasts, 6.5% neutrophils, 5.5% eosinophils, 1.5% myelocytes and promyelocytes, and 80% lymphocytes. A sternal bone marrow aspirate showed 64% blasts, 2% promyelocytes, 1% metamyelocytes, 1% eosinophils, 17% erythroid cells, and 15% lymphocytes. The blasts were large cells with a high nuclear to cytoplasmic ratio. Some blasts had multilobed nuclei and fine cytoplasmic granulation. These findings were most consistent with a diagnosis of acute myelogenous leukemia, FAB type M2. Occasional cells with cytoplasmic and nuclear characteristics of small hypolobated megakaryocytes were noted. A bone marrow clot section showed 100% cellularity with large numbers of immature myeloid cells. The patient died 1 month following induction chemotherapy of infectious complications. Archival marrow could not be amplified by PCR.

Case IV-6

This is a 16 year old male who presented in 1995 with recurrent atraumatic conjunctival hemorrhages. There was no other evidence of bleeding by history or physical examination. Laboratory studies revealed a hemoglobin of 150 g/L, a white blood cell count of 3.0×10^9/L with an absolute neutrophil count of 0.68×10^9/L, and a platelet count of 140×10^9/L. PT and PTT were normal. He was referred to the Mayo Clinic. The bone marrow biopsy showed a hypocellular marrow with mild megaloblastic changes in the erythroid series and cells with cytoplasmic and nuclear characteristics of small hypolobated megakaryocytes (Fig. 7) suggestive of myelodysplasia. The myeloid series appeared normal. Flow cytometry studies indicated less than 5% blasts. Cytochemistry revealed a normal pattern of staining with myeloperoxidase, chloroacetate esterase, and α-naphthyl butyrate esterase. The hematologic studies indicated no diagnostic abnormality. Cytogenetic studies performed on the bone marrow aspirate showed monosomy 7 in 13 of 20 metaphases. In each metaphase there was additional euchromatic material present on 9p21-22. Karyotype of circulating lymphocytes from peripheral blood found the identical 9p variation in 20 of 20 metaphases but none contained monosomy 7 (Fig. 8). Karyotype of circulating lymphocytes from both parents revealed the 9p variation in all cells from his father. A cell line derived from the patient's second cousin (not indicated in the pedigree of Fig. 1), the grandson of case II-9, who was followed at the Mayo Clinic because of a diagnosis of Friedreich Ataxia, was also found to contain the 9p variation (Fig. 8, also see Discussion).

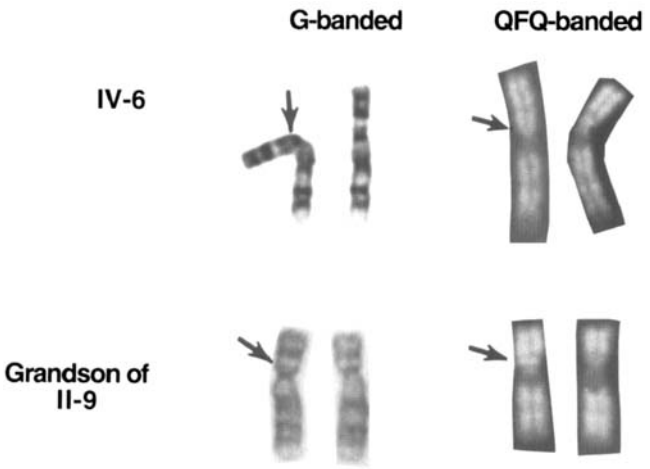

Fig. 8. Constitutional cytogenetic variation of 9p21-22 in case IV-6 and in a grandson of case II-9. Shown are preparations from circulating lymphoblasts.

Case II-3

A death certificate for this case records expiration in Arizona in 1963 at age 41 from "septicemia and pneumonia" due to a "blood dyscrasia" of 6 weeks duration, in accord with the family's recollection of a diagnosis of leukemia. Medical records and tissue samples were destroyed.

Case III-7

The son of the previous case first came to medical attention at the Bethesda Naval Hospital in 1982 at age 35. His primary symptoms consisted of arthralgias, relieved by anti-inflammatory medication. At the time of presentation, he had a white count of 2.4×10^9/L, hemoglobin of 143 g/L, and a platelet count of 110×10^9/L. His cytopenias were felt to be the result of autoimmune destruction. Six months later, the white count was 2.3×10^9/L, the hemoglobin was 150 g/L, the MCV was 102 fL, and the platelet count was 116×10^9/L. At this time the antinuclear antibody (ANA) was positive at a titer of 1:512. This was the patient's only positive ANA. No rheumatoid factor was ever detected. Seventeen months later, the patient underwent bone marrow aspirate and biopsy for persistent leukopenia and thrombocytopenia. Six months before this time the patient had an episode of easy bruising, epistaxis, and bleeding gums. The hemoglobin was 137 g/L, the MCV was 99.8 fL, the white count was 1.7×10^9/L; the platelets were not able to be counted due to clumping, but appeared to be slightly decreased based on examination of a peripheral blood film. The peripheral blood showed anisopoikilocytosis and leukopenia. Occasional giant platelets were seen. No blasts were seen in the peripheral blood. The bone marrow aspirate smear showed cells with cytoplasmic and nuclear characteristics of small hypolobated megakaryocytes.

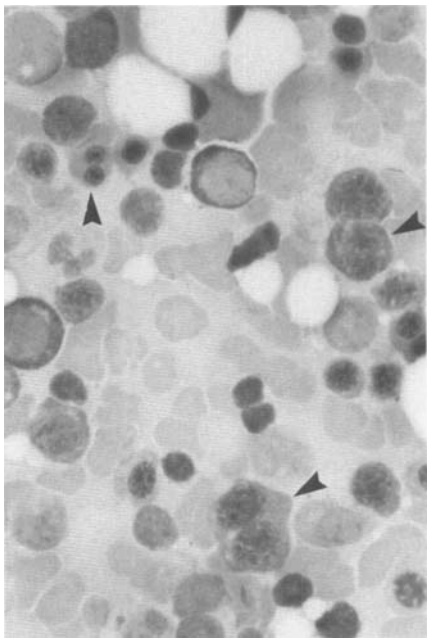

Fig. 9. Case III-7, bone marrow aspirate smear, ×500, Wright-Giemsa stain. There is a relative increase in erythroid elements with dyserythropoiesis and numerous abnormal binucleated erythroid cells (arrowhead).

There was an increase in erythroid cells relative to myeloid cells, with an M:E ratio of 0.4. The erythroid series showed dysplastic features such as binucleation, nuclear irregularity, and megaloblastic changes (Fig. 9). Some of the myeloid cells appeared to be hypogranulated. The differential cell count included 2% blasts, 4% metamyelocytes, 4% bands, 7% neutrophils, 7% eosinophils, 57% erythroid cells, and 19% small lymphocytes. The bone marrow core biopsy showed normal cellularity with a marked erythroid hyperplasia. There were numerous cells with cytoplasmic and nuclear characteristics of small hypolobated megakaryocytes. There was no increase in immature cells. The clinical and morphologic findings were consistent with a diagnosis of myelodysplastic syndrome, FAB type RA (refractory anemia). Subsequent medical information is not available. Civil records record his death 5 years later, and family members attribute it to leukemia. Due to the limited quantity of archival tissue, PCR mutation analysis was not attempted.

Case II-2

The patient, a great aunt of the proband, was 59 years old when whe presented to the University of Utah Medical Center in 1979 with persistent fever and lymphadenopathy. There was a history of chronic anemia and arteritis treated with prednisone 5 years earlier. There were no risk factors for HIV infection. At presentation her white count was 6.2×10^9/L, her hematocrit was 38.6%, and her platelet count was 261×10^9/L. A lymph node biopsy

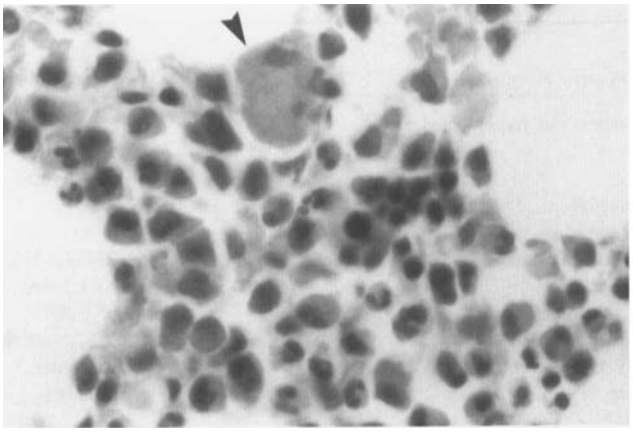

Fig. 10. Case II-2, bone marrow core biopsy, ×500, H&E stain. The specimen was poorly preserved, but a small, hypolobated megakaryocyte can be identified (arrowhead).

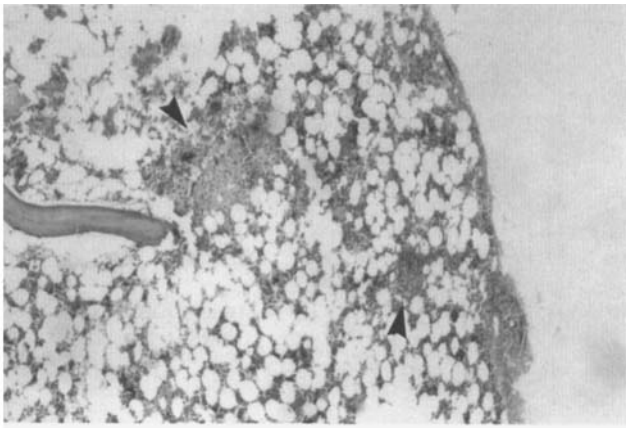

Fig. 11. Case II-2, bone marrow core biopsy, ×40, H&E stain. There are two collections of epithelioid histiocytes consistent with granuloma formation (arrowhead).

culture grew *Mycobacterium avium intracellulare;* acid-fast bacteria subsequently were cultured from her liver, retroperitoneal lymph nodes, and a tuboovarian abscess. Bilateral bone marrow core biopsies and clot sections showed normocellular trilineage marrow with good myeloid and erythroid maturation. Most megakaryocytes were well differentiated, although a rare cell with morphologic characteristics of a hypolobated megakaryocyte was noted (Fig. 10). There were also scattered collections of epithelioid histiocytes, consistent with granuloma formation (Fig. 11). Stains for acid-fast organisms and fungi were negative. The biopsy material was of insufficient quality to detect subtle dysplastic changes, and no aspirate was available. Over the next 2 years the patient responded clinically to six drug antibiotic therapy but was unexpectedly found dead at home, her head beside the bathtub. Only N-*ras* exons 1 and 2 and *p53* exon 8 could be amplified by PCR of preserved tissue, and no mutation was detected by SSCP analysis.

Case II-8

She is another great aunt of the proband and is understood by family informants to have died from leukemic complications at approximately 44 years of age in about 1974, after having undergone prior splenectomy. No records have been found.

Other Family Members

Individual I-1 died at 49 years of age in 1940 with the death certificate indicating cerebral hemorrhage. Individual I-2 died at 85 years of age in 1981 with the death certificate listing strangulated hernia. Obligate carrier II-1 is reported to be well at 72 years of age. Obligate carrier II-4 is 74 years old and carries a diagnosis of pernicious anemia; a bone marrow exam has not been performed. Obligate carrier III-11 is reported as well at 45 years of age. The only other individual with malignancy is II-7, who died at 62 years of age from hemorrhagic stroke. Her medical records confirmed a history of remote, unilateral, surgically cured breast cancer. Individual III-18 is said by family to have died from hemorrhagic stroke in his fourth decade.

DISCUSSION

The clonal blasts in AML may arise at multiple points of hematopoietic differentiation [15]. In spite of having inherited a common gene, among those family members where subtype classification is possible, at least two different subtypes are present; cases II-9 and III-1 are consistent with M2, while case IV-1 fits M7. The affected individuals also differ in other clinical features, including acuity of presentation, tumor cytogenetics, and presence of marrow fibrosis (Table I). We hypothesize that this family transmits a deficiency in a genetic locus important to the normal differentiation of multiple hematopoietic lineages. Its aberrant expression predisposes to leukemia, the affected lineage apparently depending upon which genes subsequently sustain "hits" from somatic mutation.

This is in accord with a multistep process of leukemia evolution. It is noteworthy that three obligate carriers (II-2, II-4, and III-11, the first two elderly) remain apparently unaffected. The initial step may very well require somatic mutation of the normal allele at this locus, just as is the case for other cancer syndromes inherited through mutation of tumor suppressor genes [1]. The other two large AML families [7,9] also demonstrate reduced penetrance.

A supportive line of evidence for multistep evolution of leukemia comes from studies of clonal markers in patients with AML induced to remission [16]. Clinical remission has been observed in some with the persistence

TABLE I. Summary of Affected Family Members*

Case	Age, sex	Location, year	Clinical diagnosis, duration of illness	Marrow histopathology	Marrow karyotype	Marrow mutations
II-2	59, F	Salt Lake City, 1979	Atypical mycobacterium, 3 years	Granulomas, rare hypolobated meg.		*p53* E5,6,7-NA, *p53* E8-nrml, *ras* E1,2-nrml
II-3	41, F	Flaggstaff, 1963	Dyscrasia, 3 weeks	Not available		
II-8	44, F	Pennsylvania, 1974	Leukemia, unknown	Not available		
II-9	32, F	Minneapolis, 1967	M2 AML, 1 year	Occasional micromeg., myeloid-like blasts	const.+9p22[a]	*p53* E5,6,7,8-NA, *ras* E1,2-NA
III-1	38, M	Seattle, 1975	Myelodysplasia, M2 AML, 4 years	Monocytoid-like blasts (myeloperoxidase neg./esterase neg.), absent erythroid cells, absent megs. except rare micromeg.	nrml	*p53* E5,6-NA, *p53* E7,8-nrml, *ras* E1-nrml, *ras* E2-NA
III-4	23, M	Salt Lake City, 1966	Myelodysplasia, AML, 5 years	Hypocellular, absence of erythroid cells, absence of megs., poorly diff. blasts		*p53* E5,6,7,8-NA, *ras* E1,2-NA
III-7	35, M	Bethesda, 1982	Myelodysplasia (RA), 5 years	Micromegs., dyserythropoesis, hypogranulated myeloid cells		
IV-1	21, F	Seattle, 1992	M7 AML, 4 weeks	Fibrosis, blasts appear as micromegs (rare blast myeloperoxidase pos.), dysplastic granulocytes, CD13,33,34,41 pos.	Monosomy7, t(1q:7p) trisomy8	*p53* E5,6,7,8-nrml, *ras* E1,2-nrml
IV-4	16, M	Seattle, 1993	Well donor	Occasional micromeg., dyserythropoesis	nrml	*p53* E5,6,7,8-nrml, *ras* E1,2-nrml
IV-6	16, M	Rochester, MN, 1995	Minor bleeding diathesis	Hypocellular erythroid megaloblastic changes, hypolobated meg.	Monosomy7, const.+9p22	

*The indicated age is at time of presentation (except case IV-1 where duration before presentation is indicated). The duration of illness is from onset of symptoms to time of death. meg., megakaryocytes; micromeg., micromegakaryocytes; const., constitutional; E, exon; NA, no amplification detected by PCR; nrml, normal.

[a]This patient's karyotype has been inferred from her grandson.

of the apparently leukemic clone (known as "clonal remission"). Presumably, an overtly leukemic "subclone" is ablated, but with repopulation of the not-quite-malignant, progenitor clone.

Four of the patients in this family (III-1, III-4, III-7, IV-6) presented with myelodysplasia. Eight families with multiple individuals within a sibship presenting with myelodysplasia have been described [17–23]. The absence of multigenerational transmission suggests autosomal recessive inheritance, in distinction to the autosomal dominant transmission apparent in the family we report. Interestingly, group C or chromosome 7 monosomy in cells of the marrow accompained the development of myelodysplasia in patients from all of these families. In contrast, in the family reported here, only two members (IV-1 and IV-6) were found to have monosomy 7 in the bone marrow. Although he demonstrates a dysplastic marrow, individual IV-6 remains essentially asymptomatic. IV-1 presented abruptly with M7 AML relatively late in a pregnancy in which she received routine care. The fact that she was well until this point makes it unlikely that she had a myelodysplastic phase to her illness. We thus cannot conclude that either myelodysplasia or the development of monosomy 7 are necessarily associated steps in disease progression in this family, although we cannot exclude the possibility that these events have a role in defining from what lineage the leukemia will eventually arise. We would speculate that the actions of the gene being transmitted by this family are distinct from the events leading to myelodysplasia with monosomy 7 in those other families.

The postulated gene may therefore affect multiple lineages through one of three potential mechanisms: (1) it could act early in stem cell differentiation; (2) it could be commonly expressed in all differentiated lineages; or (3) it could affect the marrow microenvironment (producing a so-called "field effect" [24] through tissue architecture or diffusible growth factors) and be expressed in cells other than the blasts. In support of the first of these possibilities, it has recently been demonstrated [25] that the acquired cytogenetic abnormalities in AML are frequently detectable in pluripotent (CD34+/CD38−) cells. The insignificant presence of other malignancies in this family implies that the effects of this gene are limited to hematopoiesis.

Features common to affected family members include

the poor response to chemotherapy, the presence in the marrow of hypolobated micromegakaryoctes and dyserythropoiesis, and the absence of detectable somatic *p53* or N-*ras* mutations. Two affected family members share cytogenetic variation of chromosome 9p21-22.

Germ line *p53* mutations previously have been excluded [2] in some familial chronic and acute lymphocytic leukemia and in one of the other AML kinships [originally reported in 9]. *p53* and N-*ras* mutations are present in 25 and 50% of AML, respectively [reviewed in 26], and are common in malignancy. Their undetectable presence in diseased marrow here suggests that these may be relatively late events in leukemic progression. Indeed, the evolution of myelodsyplasia to AML has been associated with activating mutations of N-*ras* [27]. In chronic myelogenous leukemia, *p53* mutation can be absent during the chronic phase and emerge during blast crisis [28]. We caution that the mutational analysis that we preformed is limited by the poor quality of specimen available from some of the patients, the fact that not all exons were examined, and the incomplete sensitivity of the SSCP method.

The family's common histopathologic changes are prominent in the preleukemic marrows of patients III-7 and IV-6, who presented with myelodyplasia. They may serve as an early marker of this gene's phenotype. It is significant then that the marrow of individual II-2, who was a 50% risk for inheriting the leukemia gene, demonstrates hypolobated megakaryocytes. Although she did not develop leukemia, she suffered from extrapulmonary infection with *Mycobacterium avium intracellulare.* Such opportunistic infection, most commonly limited to individuals impaired in cellular immunity, leads us to speculate that the effects of this gene are responsible. While her prior treatment with corticosteroids could be somewhat of a risk factor for this infection [29], a report of a woman with incipient meylodysplasia who also developed disseminated extrapulmonary *mycobacterium avium intracellulare* infection while being treated with corticosteroids for arthritis may be relevant [30].

Individual IV-6 demonstrates constitutional euchromatic variation of chromosome 9p21-22. The same variation was found in a cell line obtained from the grandson of individual II-9. It can therefore be deduced that case II-9 must also have carried this constitutional cytogenetic variation. This raises the possibility, falling short of statistical significance, that the 9p21-22 cytogenetic variation represents a polymorphism that segregates with this family's leukemia gene. Three candidate loci for leukemia in this region are the tandemly linked cyclin dependent kinase inhibitors p16 and p15, the translocation breakpoint gene AF-9, and the type I interferon gene cluster. Mutation of p16 and p15 have been found in AML and other hematologic malignancy [31]. Translocations involving AF-9 (with *HRX*) are found in some cases of AML [32]. Translocation and deletion within the interferon gene cluster is assoicated with AML [33,34]; moreover, there is evidence that the type I interferons contribute to host defense against infection with *Mycobacterium avium intracellulare* [35].

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